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Clinical Findings and Prevalence of the Mutation Associated with Primary Ciliary Dyskinesia in Old English Sheepdogs

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Background: Primary ciliary dyskinesia (PCD) is generally a recessively inherited disorder characterized by dysfunction of motile cilia. A mutation in a new causative gene (*CCDC39*) has been identified in the Old English Sheepdog (OES).

Objectives: To describe the clinical findings and the molecular changes of affected dogs and estimate the worldwide prevalence of the mutation in a large cohort of OES.

Animals: 578 OES, including 28 affected and 550 clinically healthy dogs.

Methods: This retrospective study reviewed the data of OES diagnosed with PCD and OES tested for the mutation. Clinical data including results of physical examination and further investigations were obtained on 11/28 dogs. *CCDC39* expression was assessed by qRT-PCR and Western blot analysis in affected dogs and healthy dogs. DNA was extracted on 561/578 dogs and a genetic test by Taqman technology was developed to genotype the *CCDC39* mutation in these dogs.

Results: Clinical findings were recurrent nasal discharge and cough, pyrexia, leucocytosis, and bronchopneumonia. Ultrastructural defects were characterized by central microtubular abnormalities and decreased number of inner dynein arms (IDAs). Molecular analysis revealed a reduced expression of *CCDC39* RNA and an absence of CCDC39 protein in affected dogs compared to healthy dogs. The mutation was more frequent in nonrandomly selected European OES population with a higher proportion of carriers (19%) compared to non-European dogs (7%).

Conclusion and Clinical Importance: *CCDC39* mutation is dispersed in a worldwide population and is responsible for PCD in this breed. Genetic testing might enable control of this disease.

Key words: Bronchopneumonia; *CCDC39*; Ciliary dysfunction; Dog; Genetic.

Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder characterized by abnormally functioning cilia. The main clinical signs are recurrent or persistent respiratory infections because of the lack of effective ciliary motility causing abnormal mucociliary clearance.[1] The dysfunction of the monocilia of the embryonic node might also lead to the randomization of the left-right body asymmetry and transposition of the thoracic and abdominal organs in 50% of the cases called the Kartagener's syndrome.[2] Male fertility can be impaired because of defects of the spermatozoa flagella. Disorders such as hydrocephalus or serous otitis have occasionally been reported.

This condition has been described in human beings as well as in different animals and in particular within 19 breeds of dog.[3–8] Dog cases reported often focus on 1 individual from a specific breed, but sometimes littermates are affected[9–11] or different cases are segregating within the same breed.[7,12] This genetic disease is usually inherited in an autosomal recessive mode.[7,12]

Motile cilia are composed of a microtubule backbone, the axonema, consisting of 9 microtubule doublets surrounding a central pair. Inner and outer dynein arms extend from each outer microtubule doublet and generate the force needed for motility in an ATP-dependent process. Ciliary dysmotility or immotility is often associated with ultrastructural defects of the cilia such as total or partial absence of the outer dynein arms (ODAs), inner dynein arms (IDAs), or both, defects of radial spokes or nexin links, and general axonemal disorganization with microtubular transposition.[1] There are a large number of genes involved in the molecular complexity of the cilium[13] and until now only a few have been implicated in the etiology of PCD mostly through human family based linkage studies or by candidate gene approach with information from genes known to cause specific ultrastructural and functional defects in model ciliated species such as *Chlamydomonas*[14] or mouse.[15]

Abbreviations:

BALF	bronchoalveolar lavage fluid
DNA	deoxyribonucleic acid
IDAs	inner dynein arms
ODAs	outer dynein arms
OES	Old English Sheepdog
PCD	primary ciliary dyskinesia
PCR	polymerase chain reaction
qRT-PCR	quantitative real-time PCR
RNA	ribonucleic acid
SNP	single nucleotide polymorphism
TEM	transmission electron microscopy

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The first objective of the study is to detail several PCD clinical cases segregating within a pedigree of Old English Sheepdogs (OES). Thanks to this pedigree a new gene involved in the motility of cilia was discovered: this *CCDC39* gene once mutated is responsible for PCD both in OES and in human.[16] The other objectives of this study are to gain further information on the molecular consequences of the *CCDC39* mutation in the dog and to describe the prevalence of the mutation in a large cohort of OES.

Materials and Methods

In total, 578 OES were involved in this study, 28 affected and 550 clinically healthy dogs. Among the affected dogs, we obtained complete clinical data for 5 of them and partial clinical data or postmortem data for 6 others. The remaining 17 affected dogs had compatible clinical signs and were identified based on interviews with breeders and owners. These dogs were only included in the pedigree analysis as no DNA was available for them.

Fifteen healthy dogs were collected from the 2 kennels where affected dogs were identified. Dogs were considered as healthy based on physical examination, if they were older than 7 months, and if they had no persistent history of signs of respiratory disease. They were primarily used for the genotyping and the identification of the mutation. For the remaining 535 healthy dogs, 427 were collected during an exhibition show or directly sent by breeders or owners and 108 dogs came from a US canine DNA bank.[a] DNA samples of American dogs were randomly collected from various American Kennel Club registered dogs and were not collected for the study. All these samples have been used to study the worldwide prevalence of the mutation within the breed.

Pedigrees of affected dogs and details of their littermates and parents were obtained when available. Litter analysis of the dogs was performed to confirm the recessive mode of inheritance.

The 5 affected dogs for which we obtained complete clinical data were investigated at the small animal clinic of the University of Liège. Blood samples for hematology, biochemistry, and DNA extraction were obtained from all of them. Thoracic radiographs (left lateral, right lateral, and dorso-ventral views) were taken.

Rhinoscopy and bronchoscopy were performed on 3 of these dogs under anesthesia with the animals breathing spontaneously. Bronchoscopy[b] allowed macroscopic evaluation of pharynx, larynx, and trachea as well as primary and secondary bronchi of each lung lobe. Bronchoalveolar lavages were cultured for bacteria and examined cytologically. Cell cultures were performed on nasal and bronchial mucosal biopsies according to previously described method (ciliogenesis).[3] The percentage of transverse sections of cilia showing abnormalities was determined from each biopsy on the basis of the examination of 121–249 cilia.

A semen sample obtained from 1 dog by manual ejaculation was immediately analyzed by light microscopy. Sperm cells were fixed and stained with a Diff-Quick protocol.

For genotyping, DNA was extracted from 1 mL of total blood with EDTA. To identify the mutation, SNP genotyping of 5 affected and 15 healthy dogs was performed on canine array. [b,16] Briefly, genome-wide association study allowed the identification of one and only 1 region of homozygosity shared by all cases on chromosome 34 and absent in healthy animals indicating a simple recessive disease. Statistical analysis demonstrated a highly significant association ($P < .001$ genome-wide). This shared segment was 15 Mb long containing 151 annotated genes. By mining 2 cilioma databases 10 candidate genes were selected as they are implied in the structure or in the function of the cilium. Sequencing the coding exons and the intron-exon boundaries of 6 of them in 1 affected dog, 1 obligated carrier, and 1 healthy dog allows the identification of one single mutation associated with a loss of function of the *CCDC39* gene. A premature stop codon predicted to truncate 90% of the protein in the affected dogs. No other disruptive mutation was detected. Furthermore, 10/10 additional cases were homozygous for the mutation, 10/10 obligated carriers were heterozygous, whereas the mutation was not found in 80 healthy animals from 9 other breeds.[c]

Small pieces of bronchial tissues from affected and healthy animals were flash-frozen. Total RNA was extracted using commercial RNA purification kit.[d] cDNA was synthesized using commercial kit[e] and a mixture of random hexamer and oligo (dT)20 primers according to the instructions of the manufacturer. Real-time PCR was performed with the 7900HT Real-Time PCR system.[f] *CCDC39* and internal control genes were detected by 20 ng template cDNA, 1× ABsolute Blue QPCR SYBR Green Mix[g] , and 0.1 μM primers in a total volume of 15 μL with a PCR condition of 15 minutes at 95°C and 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute, followed by a disassociation curve analysis. Each assay was duplicated and analyzed with commercial software.[h] We used 2 amplicons per gene. Data were normalized using 3 housekeeping genes (HPRT1, ACTB, and HMBS).

Crude protein extracts of testis, bronchial tissues, and lung were obtained after disruption and homogenization with a tissue lyser[i] and total protein concentrations determined by a colorimetric test.[j] Fifteen milligrams were diluted in 15 mL final volume (16 SDS gel-loading buffer) and loaded on a 5% stacking—10% resolving Tris-glycine SDS-Polyacrylamide gel. Proteins were separated by electrophoresis at 120 V–250 mA during 3 hours, visualized by Coomassie blue staining, and electro-transferred overnight to Hybond P PVDF membranes.[k] Membranes were blocked with 5% skim milk in PBS-Tween 20 followed by incubation with polyclonal CCDC39 antibody[l] (1 : 200) in a total volume of 3 mL for 1 hour 30 min. After washing, the specific signal was detected by 1 : 1,500 conjugated secondary rabbit antibodies[m] following the instructions of the manufacturer. Membranes were blocked with 5% skim milk in PBS-Tween 20.

A Taqman assay was developed to genotype the *CCDC39* mutation, with AGCATTTTAAGATCATTGCTGAGAGAGA as up PCR primer and TCCCGTATTGAAGTCATTTCATTT TCCA as down PCR primer, and CTGGGA**T**GAATAAAG GATGAAAT (Affected), CTGGGA**C**GAATAAAGGATGAA AT (Wild type), and TCATCCTTTATTCATCCCAG (quencher) as Taqman probes. Reactions were carried out on 7900HT instrument[e] by standard procedures. The percentage of genotyping as well as the sensitivity and the specificity of the Taqman assay were determined by comparison between Taqman genotyping results and traditional sequencing method on 191 dogs.

Prevalence of the mutation among OES was determined by including most of the OES samples collected. In order to minimize bias that could lead to an overestimation of the prevalence of the mutation within European population, dogs used in the initial genotyping study as well as dogs that were closely related to the aforementioned were not included in the prevalence study. The percentages of negative, carrier, and affected dogs were also calculated. Dogs were recorded as negative (homozygous wild type) if only cytosine was present at codon 96 in exon 3 of the *CCDC39* gene. Dogs were recorded as carrier (heterozygous) if both cytosine and thymine were present in that position and samples were recorded as affected (mutated homozygous) if only thymine was present in that position. We also collected data regarding country of origin of the dog (breeder localization).

Results

History and Clinical Findings

Among the 5 initially identified dogs, 3 (dog 1–3) were 8-month-old females from the same litter containing 5 puppies, 1 (dog 4) was an 8-month-old male from another litter containing 9 puppies also including 2 other affected siblings. The last 1 (dog 5) came from another litter including 8 puppies and was diagnosed at 3 years old and 10 months.

All dogs presented few days after their birth recurrent chronic mucoid to mucopurulent bilateral nasal discharge, moist productive cough with intermittent episodes of dyspnea, pyrexia, and anorexia, resolving only transiently with various treatments (antibiotics, mucolytics, and nebulization). All dogs were in good body condition, bright, alert, and responsive.

On presentation, clinical signs included hyperthermia, tachypnea, bilateral mucopurulent nasal discharge, and positive tracheal pinch, inducing moist productive cough. Thoracic auscultation revealed pulmonary crackles in some dogs. Complete blood count revealed leukocytosis in all dogs, mainly neutrophilic and monocytic. Packed cell volume and biochemistry did not reveal abnormalities in any dog.

Further Investigations

Thoracic radiographs revealed severe bronchointerstitial pattern in all affected dogs, associated with cranial bronchiectasis, alveolar pattern in cranial lung lobes compatible with bronchopneumonia or lung consolidation. The cardiac silhouette and the gastric fundus were in dextroposition on the ventro-dorsal projection because of situs inversus in 1 dog. Situs inversus was also identified on 2 additional affected dogs.

Direct rhinoscopy revealed hyperemic mucosa, moderate to large amounts of mucopurulent secretions, and moderate turbinate lysis. During bronchoscopy, hyperemic mucosa and large amounts of foamy mucoid secretions were observed along all the upper and lower airway tracts. Bronchoalveolar lavage fluid (BALF) analysis was compatible with acute bronchopneumonia in 3 cases and BALF culture were positive for different microorganism.

Spermogram conducted on 1 affected male revealed reduced number of sperm cells (78×10^6, normal value: $>200 \times 10^6$) with reduced motility suggesting oligoasthenospermia. Sperm cells morphologic primary anomalies were identified in 55% including narrowed midpiece (32%) and shortened flagellum (22%) (Fig 1). 28% of sperm cells were normal whereas 19% of them had secondary anomalies.

Ultrastructural analysis of the ciliary epithelium of 3 affected dogs revealed large numbers of central microtubular pair abnormalities (37.4, 37.6, and 22.5%, respectively) compared to control dogs. The most prominent defect included absent or eccentric central pairs and occasional central displacement of

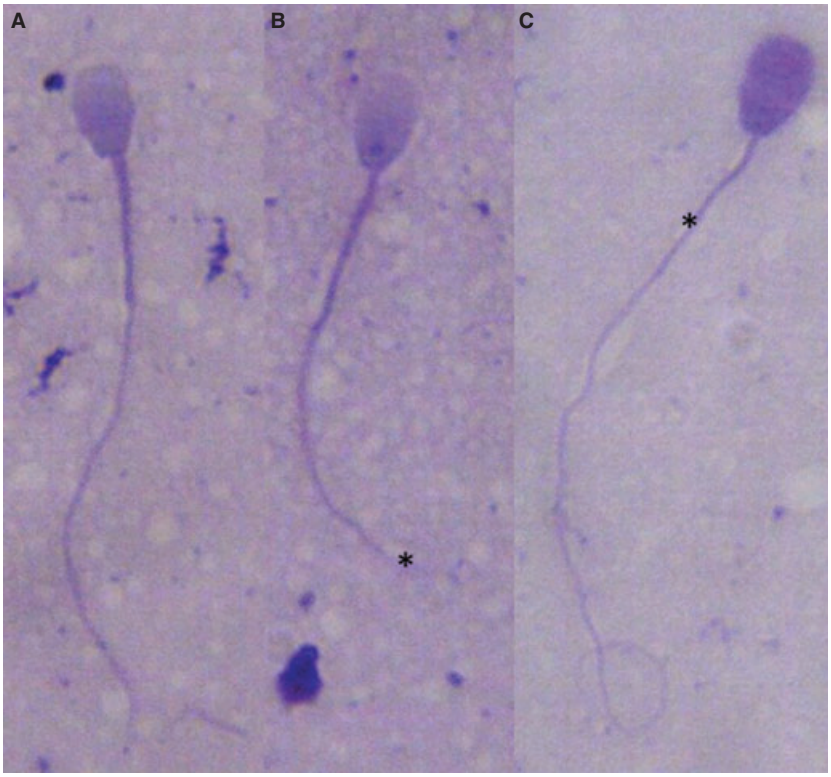

Fig 1. Spermatozoa from an affected dog after Diff-quick protocol staining, showing normal morphology (A), shortened flagella (B), and narrowed midpiece (C).

outer doublets. Electron microscopy also revealed reduction in the mean number of IDAs and high percentage of total secondary ciliary abnormalities (72.9, 61.5, and 52.3%, respectively) were also observed. Ciliogenesis of 1 dog confirmed central pair anomalies with eccentric location associated to peripheral doublet transposition into the middle of the axonema (Fig 2).

Pedigree Analysis

The pedigrees of all affected dogs were analyzed at least up to 4 generations (Fig 3). Pedigree analysis of the affected litters indicated a common closest ancestor to all parents and all affected dogs. This female lived in the eighties. This common ancestor won several dog shows and was used in Europe for breeding purpose. Interestingly, both parents of this female are coming from American line of OES. Litter analysis showed 28 affected dogs of 66 (42%) total offspring in 13 litters from phenotypically normal parents. Using Lenz-Hogben correction for bias of ascertainment, Chi-squared analysis confirmed that the litter analysis was consistent with a fully penetrant simple autosomal recessive mode of inheritance ($\chi^2 = 0.74$, $df = 1$, $P = .3871$).

Fig 2. Electron microscopy of respiratory cilia from an affected OES showing the absence of IDAs (1) in all ciliary sections, and axonemal disorganization with mislocalized peripheral doublet and displacement of the central pair (2). Magnification of the axoneme showing a normal ciliary axonema (A), isolated absence of IDAs (B), and axonemal disorganization with mislocalized peripheral doublet and displacement of the central pair (C), suggesting a loss of nexin links and radial spokes.

Molecular Analysis

qRT-PCR analysis revealed a marked reduction in *CCDC39* mRNA within bronchial tissue from affected dogs compare to control dogs (Fig 4).

Western blot analysis revealed the presence of the CCDC39 protein in tracheal and bronchial samples from healthy patient with the expected size of 110.4 kDa, whereas the protein was not present in tracheal and bronchial samples from affected dogs (Fig 5). This confirms the functional impact of the mutation with a complete loss of the protein in affected individuals.

The Taqman assay developed to genotype a large number of individuals to assess the prevalence of the mutation in OES population showed a good genotyping rate (98.45%) and a specificity and sensitivity of 100% by comparison with traditional sequencing method.

Prevalence of the Mutation

In total, the 561 OES DNA samples collected originated from 26 countries; 446 European samples from 22 countries, 110 samples from North America, and 5 samples from other countries. Forty-eight dogs were not included in the prevalence study because of close relationship with known affected dogs. Within these dogs, the proportion of negative dogs (wild type homozygous), carriers (heterozygous), and affected dogs (mutated homozygous) were 27% (13/48), 50% (24/48), and 23% (11/48), respectively. Among the remaining 513 dogs, the proportion of negative dogs (wild type homozygous) was 81% (321/398) in the European population and 93% (107/115) in the other countries. The proportion of carriers (heterozygous) were respectively 19% (76/398) and 7% (8/115), whereas affected dogs (mutated homozygous) were only identified in the European OES population (1/398; <1%). The prevalence of the mutation within European dogs and non-European dogs was 10 and 3.5%, respectively. Table 1 summarized the number of wild type homozygous, heterozygous, and mutated homozygous per country.

Discussion

This study focused on the clinical findings observed in OES diagnosed with PCD and on the prevalence of the causative mutation within a large population of OES. As expected, the most common clinical signs were nasal discharge, cough, pyrexia, bronchopneumonia, and leukocytosis. A subset of affected dogs also showed organ laterality defects. The final diagnosis was achieved through electron microscopy study and revealed an uncommon phenotype for PCD patients, characterized by central microtubular abnormalities and reduced number of dynein arms. The molecular analysis reported in this study confirmed the functional impact of the mutation with a reduced expression of *CCDC39* RNA and the absence of the

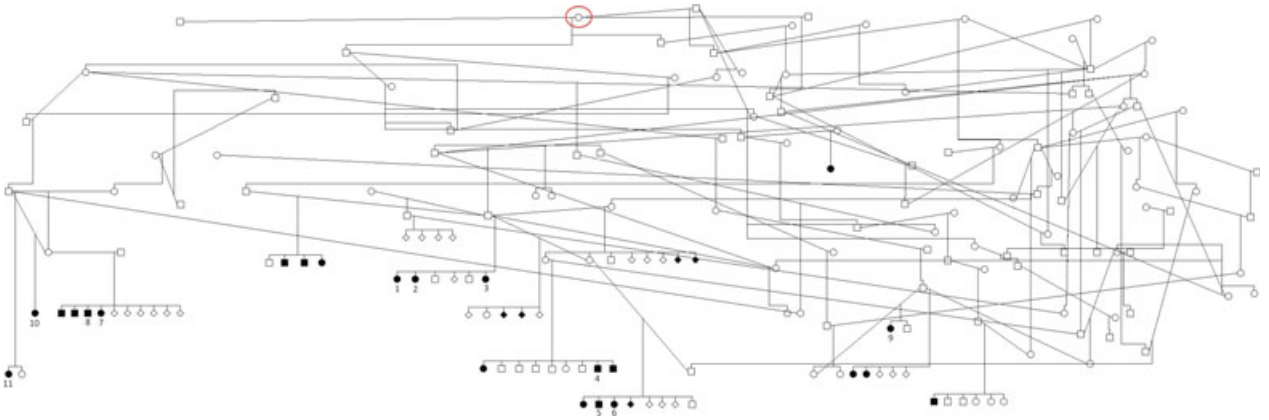

Fig 3. Pedigree of affected OES including 13 affected litters and 28 dogs with compatible clinical signs. The pedigree analysis was compatible with an autosomal recessive inherited pattern. All affected dogs and all their parents (obligated carriers) were at least linked once with the same common ancestor bitch (circled). Dogs 1–11 are dogs subsequently confirmed with the homozygous mutation in CCDC39.

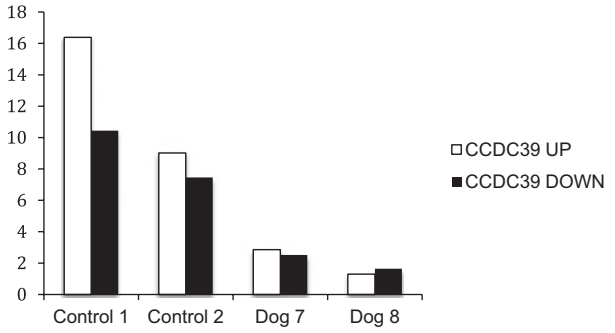

Fig 4. qRT-PCR showing marked reduction in *CCDC39* expression within bronchial tissue from 2 affected dogs compared to 2 control dogs. Two different amplicons were used.

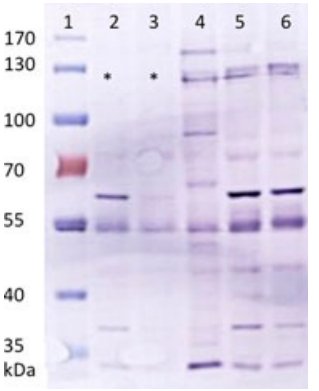

Fig 5. Western blot showing the presence of the CCDC39 protein in tracheal, bronchial, and testicular samples from healthy patient with the expected size of 110.4 kDa and the absence of the protein in tracheal and bronchial samples (*) from affected dog. (1) Colored Ladder, (2) Bronchi (Affected), (3) Trachea (Affected), (4) Testis (Healthy), (5) Bronchi (Healthy), and (6) Trachea (Healthy).

protein in affected OES. A main finding of this study was a heterogeneous worldwide distribution of the mutation. Indeed, *CCDC39* mutation was more prevalent in the European OES population compared to non-European dogs. However, European dogs were not randomly selected which might contribute to an overestimation of the mutation prevalence in this subset of dogs.

PCD is a rare, heterogeneous group of inherited structural or functional abnormalities of cilia. It occurs in people with a frequency varying from 1/15,000 to 1/20,000.[1,17,18] There is no sex, racial, or geographic bias but certain isolated communities might have a higher prevalence of PCD because of inbreeding. Therefore, it is not surprising that inbred population such as dog breeds tend to develop this disease and that PCD has been reported in more than 19 different breeds[3–8] and PCD might even occur more frequently but may not be correctly diagnosed nor publicly reported.

As genetically heterogeneous PCD might vary in severity and the organ systems affected according to the gene implicated. In this pedigree, young onset, constant rhinosinusitis, and bronchopneumonia with recurrent acute episodes are typical of PCD as the ineffective ciliary beat results in an impaired mucociliary clearance. All the affected dogs had chronic bilateral nasal discharge and productive cough that had started few days after their birth as expected for a hereditary defect. The early onset is a key feature of this disease, but affected dogs might be misdiagnosed as cases of aspiration pneumonia, or neonatal respiratory virus infection and might contribute to an underestimation of the disease. All dogs were bright with only 1 pyrexic despite the severe respiratory disease. The hematologic changes were typical of recurrent airway infection and were characterized by neutrophilic and monocytic leukocytosis present in 4 of 5 dogs. Thoracic radiographs revealed severe changes in all cases with typical bronchopneumonia lesions affecting the cranial and middle lung lobes. Bronchoscopy and bronchoalveolar lavage confirmed bronchopneumonia. Four of 5 affected dogs

Table 1. Number of dogs and percentage of wild type homozygous, heterozygous, and mutated homozygous in 561 dogs from 26 different countries. Mutated homozygous were identified in 5 countries (Belgium, Denmark, France, Spain, and United Kingdom).

Country	Dogs Number	Healthy	Carrier	Affected
Austria	5	5		
Belgium	67	42 (63)	21 (31)	4 (6)
Canada	2	2		
Croatia	4	2 (50)	2 (50)	
Czech Republic	7	4 (57)	3 (42)	
Denmark	32	19 (59)	11 (34)	2 (6)
Estonia	2	2		
Finland	23	18 (78)	5 (22)	
France	74	54 (73)	17 (23)	3 (4)
Germany	28	27 (96)	1 (4)	
Greece	5	5		
Hungary	3	2 (67)	1 (33)	
Ireland	10	4 (40)	6 (60)	
Italy	11	10 (91)	1 (9)	
Japan	2	2		
Netherlands	27	22 (81)	5 (19)	
Norway	10	10		
Poland	12	9 (75)	3 (25)	
Portugal	1	1		
Russia	5	5		
South Africa	3	3		
Spain	24	16 (67)	6 (25)	2 (8)
Sweden	14	12 (86)	2 (14)	
Switzerland	27	23 (85)	4 (15)	
United Kingdom	55	42 (76)	12 (22)	1 (2)
United States	108	100 (93)	8 (7)	
Total	561	441	108	12

had bronchiectasis lesions suggesting continuous progression of the disease in older dogs. In human, this disease typically progresses to bronchiectasis during late childhood or early adulthood and can ultimately cause chronic respiratory failure.[18]

The diagnosis of PCD was strengthened by the identification of several cases of situs inversus. Situs inversus totalis, the complete transposition of the thoracic and abdominal organs, occurs in 50% of PCD cases in human and occurs in dogs.[6,8,19] Situs inversus is an incidental finding which causes in the majority of cases no clinical problems. It is because of dismotility of cilia on the embryonic node, responsible for visceral orientation. The result is a visceral orientation determined by chance, explaining the 50% incidence.[2] We have identified situs inversus in one-third of these OES cases (data not shown). Discrepancy between this result and the expected 50% is most likely because of the small number of dogs studied.

The results of the semen analysis of 1 affected dog were typical of PCD with marked reduction in motility and high percentage of sperm cells morphologic primary anomalies visible under the light microscope and affecting sperm flagella. Semen analysis might contribute to the PCD diagnosis. However, the young onset of the disease makes its usefulness less obvious in affected puppies.

The diagnosis of PCD was definitely confirmed by defective ciliary ultrastructure under TEM. Those defects included loss of IDAs, displacement of the central pair of microtubules, and some peripheral doublets displaced centrally suggesting loss of radial spokes and nexin links. This ultrastructural phenotype is quite unusual as most of affected human beings and dogs show a loss of ODAs. It is worth mentioning that the ultrastructural findings identified in affected dogs were similar to those reported by Randolph et al, in 2 OES siblings.[11]

Identifying the genes responsible for PCD is challenging because of locus and allelic heterogeneity. Currently, PCD-causing mutations have been identified in 20 human genes[15,20–25] encoding a variety of axonemal components such as dynein arms, radial spoke, or dynein regulatory complex. A typical and common cause of motility defects is a reduction or loss of axonemal dyneins which provide the motility to the cilia, because of a mutation in one of the component of dynein arms (heavy, intermediate, or light chains). Each causative gene can be associated with particular ultrastructural ciliary defects and it is possible that a mutation in the orthologue genes might lead to the same ultrastructural defect in specific dog breeds. Therefore, careful ultrastructural analysis should be a first step to determine the best candidate genes and unravel eventually the genetic basis of PCD in various dog breeds.

These 20 genes only explain 50% of human PCD cases, more genes need to be identified. The dog is an ideal animal model to identify new causative genes.[26] First, it is a spontaneous model of the disease. Second, the ciliary structure is highly conserved across species and finally, purebred dogs represent populations with little genetic diversity that have arisen over a short period of time making genetic dissection potentially easier in dogs than in human. Indeed, this study has shown that only few dogs are required to identify a locus significantly associated with a recessive monogenic disease. Some other genetic studies in dogs have lead to the identification of genes responsible for the corresponding monogenic disorder in human[26,27] demonstrating that the dog is a useful model to elucidate genetic defect, especially monogenic recessive defect.

The functional impact of the mutation has been definitely confirmed by qRT-PCR and Western blot analysis showing reduced gene expression and absence of the corresponding protein in affected tissue compared to healthy individuals.

CCDC39 has been the first gene identified in canine PCD cases allowing for the development of a genetic test specifically designed for OES. This test not only gave us an idea of the prevalence of the mutation within the breed but also allowed to start a breeding program detecting silent carriers and preventing the birth of affected individuals. A Taqman assay was developed for this purpose with excellent sensitivity and specificity. The mutated allele was found both in European and in American OES, suggesting a worldwide distribution. However, the mutation has a higher frequency among European dogs with 19% heterozy-

gous individuals and a few homozygous only detected in European countries. The mutation shows a lower frequency in North America where we have tested more than 100 individuals with only 7% of heterozygous. The discrepancy between European and American populations might be partly explained by the nonrandom sampling of European OES. We tried to correct the nonrandom sampling for European OES by removing dogs used for the original genotyping study and dogs that were closely related to these affected dogs. However, European canine samples were not collected in a blind fashion as the American dogs. It is possible that breeders already exposed to the disease were more interested by a genetic test than other breeders inducing an overestimation of the mutation within the European OES population. This recruitment bias represents one of the limitations of our study.

The higher frequency in the European population might also be because of a founder effect as a result of the intensive use of a few champion dogs in Europe. Indeed, we were able to show that all affected dogs and their parents were at least related once to the same common ancestor. This could illustrate the negative impact of an intensive use of a champion dog responsible for spreading a rare variant within a breed. However, this finding does not confirm her as a carrier of the defective gene, but identifies the minimum age of the defective gene in the population studied. Interestingly, both parents of this female ancestor are coming from an American line of OES at the same period that Randolph et al[11] described the 2 affected OES with identical ciliary ultrastructural changes in the United States, suggesting that this genetic defect might segregate in the breed for at least 30 years. Because of the higher prevalence of the mutation in Europe, we recommend the systematic use of genetic test for avoiding breeding carriers and the birth of affected dog with associated economic loss for breeders.

In conclusion, this study reports the clinical manifestations of PCD on a significant number of OES. *CCDC39* is the first gene identified in dogs with PCD, and this study showed that the mutation of this gene is frequent in the OES population, especially in Europe. Furthermore, this study allowed the development of a simple genetic test (Taqman assay), which is currently available for breeding purpose.[n] The frequencies of the mutation reported in this study will need to be updated based on worldwide genetic screening of breeding populations. Finally, we believe that this approach might be used for PCD in other breeds to identify underlying genetic defect and should encourage us to collect data on all dogs affected by this disease.

Footnotes

[a] Canine DNA bank from National Human Genome Research Institute, NIH

[b] Paediatric bronchoscope, external diameter 4.8 mm, Fujinon EB-410S videoendoscope, ONYS

[c] Affymetrix v2.0 Canine array, Santa Clara, CA

[d] Canine DNA bank for University of Copenhagen

[e] Promega Maxwell16 Total RNA Purification Kit, Madison, WI

[f] Invitrogen SuperScript III First-Strand Synthesis SuperMix for qRT-PCR, Merelbeke, Belgium

[g] Applied Biosystems, Foster City, CA

[h] Thermo Fisher Scientific, Aalst, Belgium

[i] Biogazelle NV qBase plus software, Zwijnaarde, Belgium

[j] Qiagen Tissue lyser system II, Venlo, Netherlands

[k] Thermo Fisher Scientific Pierce BCA Protein Assay kit, Aalst, Belgium

[l] GE Healthcare, Diegem, Belgium

[m] Sigma-Aldrich, Diegem, Belgium

[n] Antagene. Veterinary genetic tests (http://www.antagene.com), Limonest, France

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Conflict of Interest Declaration: Anne Thomas works in Antagene, Animal Genetics Laboratory, Limonest, France, which provided the genetic test.

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